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BOOKS

A personal account of the epochal discovery that identified DNA as the genetic material

by Salvador E. Luria

THE TRANSFORMING PRINCIPLE: DISCOVERING THAT GENES ARE MADE OF DNA, by Maclyn McCarty. W. W. Norton & Company, Inc. ($14.95).

Between 1604 and 1618 Johannes Kepler published the results of his lifelong studies of the motion of the planets, based on the remarkable naked-eye observations made by his teacher Tycho Brahe. Kepler's work established the heliocentric view of the planetary system and formulated its geometrical interpretation in the laws that go by his name: Kepler's three laws of planetary motion.

Thinking about Maclyn McCarty's *The Transforming Principle*, and reflecting on the history of the discovery it describes—the DNA nature of the gene-transforming substance of the pneumonia bacillus—I was struck by an analogy between Kepler's achievement and that of Oswald Avery, Colin MacLeod and Maclyn McCarty. Both advances provided factual and logically satisfying pieces of evidence that explained many converging but confusing observations. In both cases the factual conclusions were reached before they could be explained in terms of mechanisms.

More than half a century elapsed between Kepler's proclamation of the laws of planetary motion and Newton's interpretation of them in terms of forces acting at a distance and universal gravitation. Only nine years separated the classic 1944 article by Avery's group on the DNA nature of the transforming principle and the discovery of the double-helix structure of DNA by James Watson and Francis Crick. Science moves faster in the 20th century.

Newton's gravitational theory and Watson and Crick's double-helix model of DNA had an aura of abstract finality, of all-encompassing "revelation," that put them beyond the range of normal discovery—they were the leaps with which science breaks into new states of integration. In contrast, the achievements of Kepler and Avery were ordinary science—intelligent, stupendous, but not exceptional as feats of the human intellect—and neither of them took the scientific world by storm. Kepler's laws were probably unknown to Galileo in 1632; the discovery of the DNA nature of the extract that transformed the pneumonia bacillus got a mixed reception. It was disputed by biochemists, more or less ignored by bacteriologists, and was welcomed only by those geneticists who had been flirting with thoughts of genes and DNA. The background of this discovery, the history of its reception and the personalities of the participants make a fascinating story.

Maclyn McCarty's book is a genuinely intimate and knowledgeable account of the transforming principle's discovery and its bearing on the later deciphering of the DNA double helix. Of the previously published histories of the achievement it is complemented in these respects only by René Dubos' *The Professor, the Institute, and DNA.* The styles of these two authors are as distinct as one might find in the writing of two serious scientists. Dubos' prose is sensitive, vigorous and assertive. His narrative is marked by worship of Louis Pasteur and a devoted if puzzled admiration for Oswald Avery. McCarty's book is both a dry, low-key autobiography and a precise step-by-step report of the research that led to the discovery. It is a model of sober restraint, presenting the author and his colleagues as well as their critics without sentimentality. McCarty is almost touchingly precise in assigning to each statement and recollection the degree of certainty or uncertainty with which he can warrant it.

Out of the two books—Dubos' and now McCarty's—there emerge, painted with different palettes, the personalities in the story, particularly that of Avery, who was known as the Professor to most colleagues (an appellation that was shortened to Fess among his closest associates). The McCarty book also makes it clear that the TP discovery was outside the main lines of genetic and biochemical research, at the limit of the almost pathetically primitive (by 1986 standards) technology of the time. Simultaneously McCarty illustrates in a paradigmatic way the opportunistic process of research at the frontiers of science: the shrewdness required to grasp at disparate leads and approaches.

The story of the discovery can be summarized in a few paragraphs. It began in 1928 when the British bacteriologist Fred Griffith, puzzled by the frequent occurrence of multiple "types" of pneumococci—the pneumonia bacilli—in the lungs of a single patient, wondered whether the virulent types, each of them characterized by specific reactivity with an appropriate serum, could interconvert. Griffith injected mice with a mixture of nonvirulent bacilli together with heat-killed virulent bacilli of a specific "type." Some mice died, and in their lungs Griffith found live virulent bacilli of the type that had been killed. Many careful controls persuaded Griffith that the virulent pneumococci (referred to as S, for smooth colonies) had not just been revived: the nonvirulent ones (called R, for rough) had been "transformed" to virulence and had acquired the "type" of the dead ones.

Griffith's report fell on prepared soil at the Rockefeller Institute's pneumonia laboratory. Pneumonia, in those preantibiotic days, was the worst cause of death in the industrial world. Avery, the head of the laboratory, was a grand master of pneumonia research and one of the great men of bacteriology. Together with Alphonse Dochez, Avery first had isolated the substances that surround virulent pneumococci and are responsible for their virulence. Then he had shown that each type (I, II, III, ...) is associated with a specific substance and that each substance is responsible for the reaction of a type with its corresponding antiserum. This was a critical advance in pneumonia epidemiology, one that promised the possibility of specific vaccination.

Later Avery had brought Michael Heidelberger into the pneumococcus field and together they had proved that the specific capsular substances of pneumococci are complex sugars called polysaccharides. The discovery of the capsular substances had revolutionized immunology by proving that molecules other than proteins could elicit antibody responses in animals. At this point Avery had brought René Dubos to the Rockefeller Institute to search for enzymes that degraded the pneumococcal polysaccharides—and

Philip Morrison's regular book reviews will appear again next month.

Dubos had succeeded: his enzyme specifically degraded type III polysaccharide and could protect mice from an injection of virulent bacilli.

When Avery read Griffith's report, he saw its importance and promptly got one of his colleagues, Martin Dawson, to repeat Griffith's experiments; Dawson's work confirmed Griffith's conclusions. Then Dawson and Richard Sia went further: they succeeded in proving that transformation of R pneumococci into specific S types could take place in a test tube rather than only in a living animal. This opened the way to precise experimentation. The next step, also taken in Avery's laboratory, was Lionel Alloway's finding that transformation did not require whole killed S bacterial cells: extracts of dissolved pneumococci, for example type III, could transform R cells derived from type I or II into live S cells of type III. The problem thereby became a biochemical one: the separation and identification of a "transforming principle" (TP) from the mixture of substances in the bacterial extract. Avery's name does not appear in any of the articles published by Dawson or Alloway, yet it is clear that Avery was and remained the driving force in the research.

Remarkably, no other investigative group jumped in to exploit Alloway's promising finding. At the Rockefeller Institute the TP research project moved at what by today's standards appears to have been a surprisingly leisurely pace, driven by Avery's conviction of the importance of identifying the transforming principle and constrained by the career requirements of a series of young medical researchers. Avery's laboratory was a most desirable center for the training in research of scientifically minded physicians, several of whom followed Alloway on the TP project. At times progress was also slowed by Avery's health problems, which led to a thyroidectomy for Graves' disease.

Enter finally Colin MacLeod. With him the effort to purify TP began in earnest, and yet it still progressed at a heartbreakingly slow pace. In their search for the transforming principle MacLeod and Avery methodically excluded one by one most components of their S-cell extract. Neither Dubos' enzyme, which broke down the polysaccharide, nor any of several enzymes that destroy proteins affected the TP activity. Tons of bacterial cultures had to be prepared for these tests and MacLeod invented the needed instrumentation as he went along.

By the time MacLeod left Avery's laboratory in 1941 many things had happened. For one thing, the arrival of sulfonamide drugs had made research on pneumococci less urgent if not altogether obsolete. He and Avery had to persuade themselves, as well as the institute's administration, of the desirability of continuing the study of TP. By then they had become convinced that TP must be nucleic acid—either RNA or DNA, substances then distinguishable only by uncertain chemical tests. MacLeod moved a mile and a half south from the Rockefeller Institute to the New York University Medical School. He remained in close contact with Avery but, surprisingly, did not pursue TP research in his own laboratory. It seems reasonable to assume that Avery was jealously keeping complete control of his pet project.

When McCarty replaced MacLeod, he may already have heard about the TP problem, but he was naive with respect to the substance of the work. His previous experience had been in pediatric bacteriology; still, he brought to the TP group the fresh drive and solid competence of a well-trained medical investigator. Methodically he and Avery refined the purification of TP, perfecting their techniques and pursuing every clue, such as the "stringy" appearance of the active fraction in an alcohol precipitate and the loss of activity on drying. Enzymes that degraded RNA did not affect this transform-

ing activity. Most important, they proved that the transformed bacteria and their descendants became permanent sources of more TP of the same kind. The TP reproduced itself.

By 1943 McCarty and Avery felt certain that TP was DNA—in fact, pure DNA. It was not an easy certainty: in 1943 no one knew what pure DNA really was. Geneticists, particularly chromosome experts, had expected DNA to have something to do with genes, but they were outnumbered by chemists who claimed it could not. The chemists believed DNA was only a monotonous polymer, a molecule without importance, at most a dull portion of something that was called "nucleo-protein." And McCarty still had no reliable test for DNA. Altogether the proof of the DNA nature of TP was anything but "rigorous," the term by which Horace Judson described it in his summary of the TP story in *The Eighth Day of Creation*. Yet Avery and McCarty, joined by MacLeod, decided to go public.

No pressure or competition or sudden emergence of irrefutable data precipitated the decision to publish. In retrospect it appears that at this point Avery may have decided to exert the prerogative of the experienced leader. He exhibited the assertiveness that comes from the habit of success: a willingness to impose on a still confused mass of data a certainty that is emotional as well as rational. Such a source of certainty in science is unrecognized by those who believe certainty comes only after innumerable controls and attempts to disprove. The certainty Avery exhibited is more akin to illumination, a sudden vision projecting the possibility of an intellectual leap. What happened in the TP research at this point may have been the maturing of the conviction that the transformations observed in pneumococci were not just an idiosyncratic phenomenon of bacteriological interest but a stupendous lead to the chemical basis of hereditary specificity.

The article by Avery, MacLeod and McCarty, which appeared in the February 1, 1944, issue of the *Journal of Experimental Medicine*, is cautious in its conclusions but revealing in its opening sentence: "Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters." Note the precise wording, which could have satisfied any card-carrying geneticist.

Earlier in 1943 Avery had written his brother a letter, which fortunately became part of the public record because Avery showed it to Max Delbrück, who after Avery's death insisted that it be published. In the letter, reproduced in part in the McCarty book, Avery sounds cautiously but deeply stirred: "After innumerable transfers and without further addition of the inducing agent, the same active & specific transforming substance can be recovered far in excess of the amount originally used to induce the reaction. Sounds like a virus—may be a gene....the problem bristles with implications.... It touches genetics, enzyme chemistry, cell metabolism & carbohydrate synthesis, etc."

Whether or not Avery and his colleagues were aware of it, there was something in the air at the time those words were written. Just as the classical biochemistry of energy metabolism reached its apogee, marked by Fritz Lipmann's great review article of 1941, genetics was entering new and greener pastures. In December, 1940, George Beadle and Edward Tatum had reported their first results on the biochemical genetics of the bread mold *Neurospora crassa*—the work that was soon to lead them to put forward the one-gene, one-enzyme hypothesis. And in 1943 Delbrück and I had published in *Genetics* the first demonstration that bacterial variants originate by spontaneous mutation.

Bacteriologists were then by and

large oblivious of genetics. Many of them accepted uncritically hypotheses such as one put forward by the physical chemist Cyril Hinshelwood, which ascribed heredity and variations in bacteria to complex flow equilibriums in chemical reactions. In his 1976 book on Avery and the Rockefeller Institute, Dubos, one of the most biologically minded bacteriologists, remarks that as of 1945 he "could find in the literature only a few sketchy experiments to support the view that... some phenomena of bacterial variability nevertheless probably fall within the fold of classical genetics." It is a fact that most bacteriologists did not read *Genetics*.

Yet people such as Avery, MacLeod and McCarty certainly knew or sensed what was in the making. Theodosius Dobzhansky, in the second edition of *Genetics and the Origin of Species*, published in 1941, gave an account (the first one to come to my attention) of the transforming-principle story and its possible bearing on the study of gene mutations. In the summer of 1941, at Cold Spring Harbor, Dobzhansky developed a close intellectual relationship with Alfred Mirsky. Mirsky was a Rockefeller Institute biochemist who, in 1942, gave McCarty some useful leads on handling DNA and even collaborated with him in one set of experiments. Mirsky later became one of the severest critics of the Avery group and of their conclusion that TP consisted of DNA. He was critical partly on grounds of rigorous biochemical standards and partly, perhaps, because of a grudge over his abortive participation in the research.

Thus the Rockefeller Institute must have been abuzz with talk of biochemical genetics. It is reasonable to suppose (the supposition is not contradicted by anything in McCarty's book) that the profound biological significance of their TP work may, sometime between 1942 and 1943, have become so urgently clear to Avery, MacLeod and McCarty that the realization induced even the supercautious Avery to publish what was a *fact*—even though a fact validated as much by conviction as by hard evidence, a fact less fully convincing to experimentally minded critics than Kepler's laws might have been to Huygens and Newton.

Here I shall take a closer look at the authors of the 1943 article and its fateful statement. I have known all three of them personally. It is ironical to say that Avery stood out among the three. He was a "petite," gnomelike man with piercing but kindly eyes under an enormous forehead and a bald top, which he constantly stroked while he delivered to visitors his customary recitations of the pneumococcal transformation story (McCarty refers to these discourses as the "Red Seal Records"). His professional stature was apparent, as Dubos pointed out, in his complete lack of self-importance. He was unfailingly gentle, courteous in an unaffected way, yet prim and restrained. He had a sharp, teasing wit: I was told of his habit of greeting any colleague who had published a short preliminary note with the remark, "I have seen your advertisement." One day in early 1946 he walked into the Rockefeller Institute library before lunch just as I had found in the current issue of *Experientia* an article by the French bacteriologist André Boivin reporting DNA-mediated transformation in the bacterium *Escherichia coli*. When I showed Avery the article, he read it quickly, asked me to join him for lunch, and when a few of his co-workers sat down with us he whispered to them, "We seem to have continental support."

I visited Avery's laboratory at least three times between 1943 and 1945. I was interested in the connection between the TP work and my own research on bacterial mutations, wondering if the DNA might not transform a special class of mutant bacterial cells. (I worked for a few months on this topic and quit when later work in Avery's lab made my idea untenable.) Avery insisted that first of all he had to be absolutely sure of the chemical nature of the transforming principle.

So did McCarty, who by that time was searching for further proof of DNA involvement. McCarty's personality was radically different from Avery's. Intensely serious and alien to discourses, rather shy, laconic in explanation, he was a model of the serious medical researcher.

Jollier and more easygoing than McCarty was MacLeod, whom I came to know much better when, after the TP discovery, he became actively interested in bacterial genetics. In 1946 MacLeod asked Vernon Bryson and me (I was then spending a year at Cold Spring Harbor) to lead a genetics seminar at N.Y.U., where he headed the microbiology department. This turned out to be a delightful experience with a group of bright, intensely interested young people. In those few years bacteriologists had discovered genetics—thanks to the transforming principle. Dobzhansky was encouraging his best students to work in bacterial genetics (one of them, Harriett Taylor, joined Avery in 1945). And Tatum and Joshua Lederberg had discovered mating between *E. coli* cells.

The response of biochemists to the identification of TP with DNA was more cautious. Their bias was anchored in the belief that DNA, which when degraded yields just four components—the four "nucleotides" called *A, G, T* and *C* for short—had a simple repetitive structure (*AGTC-AGTC* or some other repeated sequence). Therefore, the chemists reasoned, DNA could not serve to encode any specific genetic information.

It is remarkable that this hypothesis, which had been proposed by P. A. Levene without any clear analytical evidence, had taken root in the mind of the few biochemists interested in nucleic acids—an unquestioned assumption that reminds me of the equally unchallenged assumption of astronomers before Kepler that planets should move in circles, whether around the earth or around the sun. The circle, according to Greek philosophers, was the ideal geometric figure. Levene's hypothesis, like that of Greek astronomers, appears to have been initiated by an unconscious desire to impose an ideal order of the simplest possible kind on another aspect of nature. The "sacred rage for order" (to use words from a poem by Wallace Stevens) when carried too far can generate hidden assumptions. The unmasking of such assumptions may in turn become the key to new advances.

Apart from Levene's specific model of DNA, a major obstacle to the acceptance of Avery's conclusion by biochemists, even after more solid evidence was provided by McCarty and by other Avery associates, was the lack of a plausible DNA "structure" on which to hang the specificity required of a genetic material. Proteins were "proteic," that is, multiform in structure. Their specificity was demonstrated, for example by serological tests. DNA instead remained mysteriously amorphous in the mind of biochemists. Bacteria, particularly pneumococci, might, it was suggested, be a freak class of organisms. Even if bacterial genes contained DNA, what did this prove about genes in fruit flies or human chromosomes?

The relatively long delay between Avery's discovery and its generalization to other genetic materials encouraged all kinds of questioning. It is still a tormenting recollection in my own scientific life that I (who had been so close to the TP story, had written a detailed report about it in 1947, and had even briefly worked on it) could in a 1951 paper suggest that the genetic material of a bacteriophage might be protein—a few months before Alfred Hershey and Martha Chase reported their brilliant experiments identifying DNA as the phage genome.

Geneticists had for years been told by some of the shrewdest cytologists,

who were examining chromosomes by means of dyes more or less specific for protein or for nucleic acids, that DNA might be part of the genetic material. And yet others (besides myself) did not fully internalize the idea of the possible universality of Avery's discovery. Our hesitation is even more puzzling in retrospect when we consider that within months after the publication of the Luria-Delbrück test for mutations in *E. coli*, several genetic laboratories had taken up that lead. The failure of the Nobel committee to recognize the Avery work is particularly puzzling since one of the major experts in the chromosomal location and distribution of DNA was a member of the committee. Perhaps it was thought to be unseemly that the gene's identity should emerge from bacteriology.

The story of the fortunes of the TP discovery took a curious twist later when Gunther Stent, a molecular biologist and philosopher, suggested that Avery's discovery had failed to be appreciated because it was "premature." The doctrine of prematurity in discovery has provided a prime subject for arguments among historians of science in spite of the obvious circularity of the concept: is a discovery premature because it is not appreciated, or vice versa? More simply, does a discovery seem premature to those who missed its significance or failed to read or learn about it? The identification of TP as DNA was neither ignored nor unrecognized. It was questioned by some and embraced by others, and the universality of its impact remained uncertain. What Watson and Crick provided in 1953 was the chemical underpinning for the generalization.

Avery's work was unknown to Watson (he tells me) when, at the age of 18, he came to Indiana University as a graduate student. There he heard of pneumococcal transformation in my virology lectures and in Tracy Sonneborn's genetics course. Watson's fascination with DNA, which was both a rational and an emotional commitment (note the two key ingredients of science), dates from those days. It was this fascination that years later led him (with my encouragement and in spite of Delbrück's misgivings) to pursue the biochemistry of DNA. He ultimately stirred Crick to enthusiasm for that substance and drove the two of them to their great discovery, the DNA double helix: two complementary polynucleotide chains, each bearing complete directions for replication and each providing precise information for protein synthesis. DNA can be "transcribed" into RNA, which directs the synthesis of proteins.

This discovery stands at the center of biological science; it has both a towering finality and an aesthetic beauty that give it a stature comparable to the stature Newton's dynamics has enjoyed for the past three centuries in physics. Out of the double-helix model came a transformation of biology—the vision of a unity even more convincing than the great Darwinian synthesis. The clarifications of protein synthesis and of its regulation through control of gene action (a control itself mediated by specific protein-DNA interactions) are the high points of the new biology and now promise to solve the mystery of the development of complex organisms.

Reflecting on the prospects for further advance, one may be tempted to take an attitude of romantic pessimism: all that remains is either applications or epistemological disquisition. Such was the mood in physics around 1900—after Maxwell and Boltzmann, and just before Curie, Planck, Rutherford, Einstein and Bohr entered the picture. And so there is hope for the young biologists who dream of discovery.

Where might the "new" come from? I can think of two sources: areas that are today beyond the pale of molecular biology and areas where the current paradigm may again be found incomplete, as it was in physics. History, at least, offers the possibility that unwarranted hidden assumptions may be recognized in the present framework of molecular biology.

One area of persistent obscurity is the "cell theory." Can genes make a cell? Is the organization of a cell as a domain defined by a closed membrane fully coded in the genes, or does the membrane represent a self-priming pattern of organization? An answer to this question will require evidence for or against the possibility of synthesis of cells from subcellular elements. It may be sought by inquiring into the origin of polyphyletic cell types such as cyanobacteria or eukaryotic cells, which appear to have arisen from the merger of elements having different evolutionary origins.

The paradigm of molecular biology has already withstood several challenges that superficially seemed to bring into question its original formulation: the role of DNA as the genetic material and that of protein as the stuff of catalysis. Several viruses have RNA genomes, which are reproduced either through an intermediary DNA or directly as RNA. This, however, simply means that both kinds of nucleic acid can embody in their nucleotide sequences information capable of being copied. In fact, it now appears that a substantial portion of the DNA genome of animals probably originated with the copying of RNA into DNA sequences that were subsequently incorporated into the chromosomes. As for catalysis, the recent finding that an RNA molecule can in some instances catalyze its own restructuring does not shake the central notion that not genes themselves but only their products—mostly proteins but exceptionally also RNA—can function as catalysts for specific steps of cellular chemistry.

There is another twist beyond the simple picture of heredity based on mutation-prone but otherwise stable DNA sequences. It is the discovery that the response of the animal body to the innumerable variety of foreign substances involves a reprogramming mechanism for creating new DNA sequences—new genes—in the cells of the immune system. This discovery illustrates a subtle complexity of heredity based on DNA structure. The genetic endowment of an animal is more than a set of genes; it is a range of possibilities actuated during life by influences that selectively amplify clones of immune cells with the appropriately responding genes.

Molecular biology would be truly revolutionized by proof that substances other than nucleic acids can have a genetic role, that is, serve as molecular templates for the replication of themselves or for the production of nucleic acid chains. It has been suggested that amino acid sequences, primitive proteinlike molecules, might have had roles as templates in precellular stages of the evolution of life.

Whether a revolution in molecular biology is forthcoming, and whether any such revolution will emerge from novel findings at the molecular level or from resolution of epistemological quandaries, perhaps in dealing with the biology of the human mind, is for the future to tell. Avery and his coworkers were not after a revolution in science. They sought the answer to a specific question, the nature of TP, and it turned out to be a profoundly important answer. This is the normal path of the scientific enterprise: putting together the pieces of a puzzle whose design comes into existence only as the pattern grows.

Max Delbrück, speaking with Horace Judson, once pointed out that "science pretends that the scientist is immortal," a thought that caused him to worry lest it make scientists less human, less concerned about the mystery of human life. Is there for a biologist any true immortality other than having put into place, as Avery, MacLeod and McCarty did, a fragment that illuminates the overall pattern of the puzzle of life?